1975 CITRUS CHEMISTRY AND TECHNOLOGY CONFERENCE

OCTOBER 8, 1975

ABSTRACTS OF PAPERS

LANDMARK MOTOR LODGE
Winter Haven, Florida

U. S. CITRUS AND SUBTROPICAL PRODUCTS LABORATORY

600 Avenue S, N.W.
Winter Haven, Florida 33880

SOUTHERN REGION
AGRICULTURAL RESEARCH SERVICE
UNITED STATES DEPARTMENT OF AGRICULTURE

PREFACE

The Citrus Chemistry and Technology Conference is sponsored by the Southern Region, Florida-Antilles Area, of USDA's Agricultural Research Service to report developments in the broad areas of processing, marketing, nutrition, pollution abatement and related subjects, and to provide for exchange of information that will benefit the industry, future research and American consumers.

This report summarizes the statements of the various speakers during the Conference. Many of these reports are in the nature of progress reports and are subject to change as studies are completed. Please contact authors for latest results before using these reports as a reference.

Dean F. Davis, Area Director

PROGRAM

1975 CITRUS CHEMISTRY AND TECHNOLOGY CONFERENCE

October 8, 1975

CHARACTERIZATION OF ORANGE ESSENCE QUALITY BY GAS CHROMATOGRAPHY

E. D. Lund and W. L. Bryan
Citrus and Subtropical Products Laboratory
Winter Haven, Florida

ANALYSIS OF THE DISTILLATION RESIDUE FROM TANGERINE COLD-PRESSED OIL

C. W. Wilson and P. E. Shaw
Citrus and Subtropical Products Laboratory
Winter Haven, Florida

REDOX FUNCTIONING QUINONES IN CITRUS FRUIT

J. H. Bruemmer and B. Roe
Citrus and Subtropical Products Laboratory
Winter Haven, Florida

CITRUS LIMONOIDS: SOME RECENT INVESTIGATIONS

R. D. Bennett
Western Region
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

PROTEIN AND AMINO ACID ANALYSES OF DRY CITRUS SLUDGES FOR POULTRY FEED

R. L. Coleman and P. E. Shaw
Citrus and Subtropical Products Laboratory
Winter Haven, Florida

TEMPERATURE AND STORAGE EFFECTS ON % RETENTION AND % U.S. RDA OF VITAMIN C IN CANNED SSOJ

S. Nagy and J. M. Smoot
Citrus and Subtropical Products Laboratory
Winter Haven, Florida

GRAPEFRUIT JUICES AND QUALITY

J. H. Tatum and R. E. Berry
Citrus and Subtropical Products Laboratory
Winter Haven, Florida

MECHANICAL REMOVAL OF TRASH AND UNWHOLESOME FRUIT DURING UNLOADING

W. L. Bryan*, B. J. Anderson**, R. P. Snyder***, J. M. Miller**, and J. Jenkins**

*Citrus and Subtropical Products Laboratory
Winter Haven, Florida

**Florida Citrus Research Foundation
(Supported by the Florida Department of Citrus)
Lakeland, Florida

***Consulting Engineer
Largo, Florida

ORANGE FLAVOR EFFECTS DUE TO CITRUS ABSCISSION AGENTS

M. G. Moshonas, P. E. Shaw and D. A. Sims
Citrus and Subtropical Products Laboratory
Winter Haven, Florida

CHARACTERIZATION OF ORANGE ESSENCE QUALITY BY GAS CHROMATOGRAPHY

Eric D. Lund and William L. Bryan

Citrus and Subtropical Products Laboratory
Winter Haven, Florida

Orange essence of organoleptically unacceptable quality differed in composition from acceptable essence normally recovered from the same kind of commercial equipment. Major components of normal essence (N) and two low-quality essences (A and B) determined by gas chromatography are shown in Table 1. (Column 1 is normal essence composition, columns 2 and 3 show ratios of component concentrations in unacceptable as compared with normal essences.)

Essence compositions were determined by combining results of several GC analytical methods, each needed to establish some components. Methanol, acetaldehyde and ethanol, the most prevalent components were determined by direct injection on a Porapak column (3). Most remaining components were determined by direct injection on a column of Carbowax 20-M. Solvent interference prevented accurate determination of the more volatile constituents by this method. Ethyl butyrate, n-hexanal, l-penten-3-ol and n-amyl-alcohol were determined by injection of concentrated essence on a Carbowax 20-M or DEGS column (6). Three methods of concentrating essence were compared: a) solvent extraction with methylene chloride (6), b) solvent extraction with diisopropyl ether (2), and c) adsorption on a Porapak column with elution to a liquid nitrogen trap (4).

Some components of unacceptable essences differed by more than a factor of 2 from normal essence, indicated in Table 1, where ratios greater than 2 or less than 0.5 are underlined. Many such components are important to orange flavor (1), e.g., acetaldehyde and ethyl butyrate enhanced orange flavor, while t-2-hexenal reduced acceptability. In addition, the concentrations of n-octanal, a known flavor enhancer which can be accurately determined by direct injection on Carbowax 20-M, were significantly lower (0.5-0.6 ratio) in unacceptable essences.

Higher concentrations of some components in unacceptable essences may be caused by oxidation or hydration reactions during manufacture or storage. The three terpene alcohols, t-2,8-p-menthadien-1-ol, α-terpineol, and t-carveol can be produced by oxidation and hydration reactions of limonene. α-Terpineol, in particular, is known to contribute to off-flavor in orange juice (5). Six-carbon compounds can be produced under certain conditions by oxidation of lipids and carotenoids.

Direct injection on Carbowax 20-M may provide a simple analytical method to identify unacceptable essences, based on intermediate molecular weight components (Table 1, column 4). Although acetaldehyde and ethyl butyrate cannot be determined by this procedure, the method can indicate when concentrations of many undesirable components are abnormally high. Other column packings might narrow the solvent peak so the more volatile compounds could also be analyzed by direct injection.

LITERATURE CITED

1) Ahmed, E. M., Dennison, R. A. and Shaw, P. E. Presentation at the Citrus Chemistry and Technology Conference, Winter Haven, Florida, Ocotber 10, 1973.

2) Dinsmore, H. L. and Nagy, S. Presentation at the Citrus Chemistry and Technology Conference, Winter Haven, Florida, October 11, 1974.

3) Lund, E. D., Berry, R. E., Moshonas, M. G. and Wagner, C. J. Jr. Presentation at the Citrus Chemistry and Technology Conference, Winter Haven, Florida, October 9, 1970.

4) Moshonas, M. G. and Lund, E. D. 1971. J. Food Sci. 36, 105.

5) Tatum, J. H., Nagy, S. and Berry, R. E. 1975. J. Food Sci. 40, 707.

6. Wolford, R. W., Alberding, G. E. and Attaway, J. A. 1962. J. Agr. Food Chem. 10, 297.

Table 1. Essence composition

Compound[a]	Normal essence (N) wt. % x 10^3	Unacceptable: normal ratio A/N	B/N	Direct injection (Carbowax 20-M)
Methanol	770	0.90	0.70	
Acetaldehyde	121	0.29	0.29	
Ethanol	10,600	0.91	0.99	
Ethyl butyrate	3.9	0.32	0.52	
n-Hexanal	0.144	3.1	2.8	
1-Penten-3-ol	0.30	-	1.3	
3-Methylbutane-1-ol	1.32	0.55	1.3	x
n-Amyl alcohol	0.088	2.4	-	
t-2-Hexenal	0.073	12.0	2.5	x
n-Octanal	0.49	0.61	0.54	x
1-Hexanol	0.076	8.9	2.1	x
c-3-Hexen-1-ol	0.299	3.8	0.69	x
t-Linalool oxide	0.227	1.1	-	x
c-Linalool oxide	0.237	0.96	-	x
Linalool	2.57	0.82	1.0	x
1-Octanol	0.204	1.0	0.83	x
Terpinen-4-ol	0.242	0.79	0.64	x
t-2,8-p-Menthadien-1-ol	0.041	3.7	3.9	x
Ethyl-3-hydroxyhexanoate	6.4	0.50	0.88	x
α-Terpineol	0.284	2.0	1.4	x
t-Carveol	0.077	2.5	1.8	
Unidentified compounds (total)	1.53			

[a]Listed in order of retention time on Carbowax 20-M.

ANALYSIS OF THE DISTILLATION RESIDUE FROM TANGERINE COLD-PRESSED OIL

Charles W. Wilson and Philip E. Shaw

Citrus and Subtropical Products Laboratory
Winter Haven, Florida

Cold-pressed tangerine oil was distilled at 40°C and 1 mm Hg pressure to remove the more volatile materials including most of the d-limonene. The distillation residue was stored at 5°C to allow most of the flavonoids to precipitate. The folded oil was separated by preparative gel permeation chromatography (GPC) on Sephadex LH-20 with tetrahydrofuran as the solvent. The GPC fractions were further analysed using high performance liquid chromatography, thin-layer chromatography and gas chromatography-mass spectrometry combination.

The eluate from the Sephadex column was divided into six fractions by visual observation and by aroma (Table 1). The first three fractions consisted mostly of carotenoid pigments. The use of citrus color pigments purified by this GPC procedure for coloring poorly colored FCOJ will be reported in the 1975 Proceedings of the Florida State Horticultural Society. Most of the aroma was in fractions 4-6, with the most typical tangerine aroma in fraction 5. Fraction 5 was found to be a complex mixture of mostly oxygenated materials (Table 2). One component with an emperical formula $C_{15}H_{24}O$ possesses a fishy, mandarin-type aroma. From odor and flavor evaluations, this previously unidentified compound is believed to contribute significantly to tangerine flavor.

Table 1. GPC separation of folded tangerine cold-pressed oil.

Fraction number	Components	Color	Aroma
1	Carotenoids	Yellow	--
2	Carotenoids	Red	--
3	Carotenoids Flavonoids	Orange	Slight mandarin
4	Hydrocarbons Alcohols Carotenoids	Orange	Slight citrus
5	Carbonyls Alcohols Hydrocarbons Ethers	Yellow	Mandarin
6	Hydrocarbons Alcohols Ethers	Lt. yellow	Terpene

Table 2. Identified components of fraction 5.

Hydrocarbons	Aldehydes	Alcohols	Acetate esters	Ethers
Limonene	Citronellal Decanal Dodecanal Geranial Neral Octanal α-Sinensal	Elemol Linalool Thymol $C_{15}H_{24}O$	Citronellyl Decyl Geranyl Neryl 1,8-p-Menthadiene-9-yl	Thymol methyl ether

REDOX FUNCTIONING QUINONES IN CITRUS FRUIT

Joseph H. Bruemmer and Bongwoo Roe

Citrus and Subtropical Products Laboratory
Winter Haven, Florida

Careless handling of citrus in transit or in storage results in more rapid respiration by the fruit. In poorly ventilated trailers or bins, oxygen becomes depleted and CO_2 accumulates in the fruit. Under these conditions respiration becomes more anaerobic leading to higher levels of ethanol and other less desirable products in the juice. Even under normal conditions ripe citrus fruits respire anaerobically and produce ethanol. A transition from aerobic to anerobic appears to occur when the fruit starts to ripen and ethanol accumulates. Although the causative agents of ripening in citrus or other fruits are not known, the mitochondria are probably involved because they are the aerobic respiratory particles in the cell. As the first step in the systematic examination of the respiratory apparatus of the citrus juice cell we have examined mitochondria from immature grapefruit for ubiquinone, an electron carrier of the respiratory chain, and tested its functionality.

Juice cell particulates were separated by differential centrifugation into three fractions: cellular debris including cell walls and vesicular membranes (R_1), mitochondrial (R_2), and microsomal (R_3). Each fraction was extracted with n-heptane-acetone-benzene mixtures and partitioned between this solvent and ethanol-water to remove phospholipids. After removing the solvent the quinone fraction was chromatographed on alumina and developed by stepwise elution with increasing concentrations of diethyl ether in petroleum ether (0,2,3,5,8,12,20,25,30 and 100%). Redox quinones were separated from each fraction on Silica gel G plates in chloroform-heptane (97-3) and (80-20), and located by their effectiveness in oxidizing leuco methylene blue. Ubiquinone was quantitated spectrophotometrically from absorbance difference at 275 nm before and after reduction with $NaBH_4$.

Ubiquinone was concentrated in the mitochondrial fraction, R_2, but R_3 contained 0.8 nmoles per mg protein (Table 1). Apparently particles from "ruptured" mitochondrial sedimented in R_3. The concentration of 1.8 nmoles in citrus mitochondria is about one-half the amount reported for other plant mitochondria.

Methylene blue reducible compounds with Rf values similar to the plastoquinones of chloroplasts were detected on several Silica gel plates developed with R_2 extracts. UV spectrum of several of these "plastoquinones" had absorbance maximum (ΔA oxid-red) at 255 nm, typical for the quinones localized in osmiophilic globules. Our mitochondrial preparations contain chronoplasts with these globules as shown by electron microphotographs.

Activity of citrus ubiquinone in the respiratory sequence of succinoxidase was examined using isooctane-extracted citrus mitochondria. About one-half of the succinoxidase activity was restored by citrus ubiquinone (Co Q) in the test system (Table 2). Addition of a phospholipid fraction was required to restore complete activity in isooctane extracted mitochondria from other plant tissue, but a purified preparation (Asolectin) was not effective in restoration with citrus mitochondria. Demonstration that citrus mitochondria contain a functioning redox quinone indicates that they are similar to respiratory particles in other plant tissues. In those plants Co Q was used as an indicator of the respiratory state.

Analysis of quinones in ripe and unripe citrus fruit could indicate the respiratory states during maturation and lead to better understanding of the respiratory change in ripening fruit, and mishandled fruit for processing.

Table 1. Distribution of ubiquinone in juice cell organelles.

Preparation		Ubiquinone nmoles/mg protein
R_1	Cell debris	0.2
R_2	Mitochondrial	1.8
R_3	Microsomal	0.8

*Ubiquinone was extracted from the particulate preparation, purified by liquid-liquid partition, and determined spectrophotometrically:

$$\text{nmoles/mg protein} = \frac{3 \times \Delta \text{ A oxid-red } 275 \text{ nm} \times 10^3}{12.5 \times \text{mg protein/ml}}$$

Table 2. Coenzyme Q dependent succinoxidase in isooctane extracted mitochondria

Addition	Succinoxidase activity* Before	After
None	12.2	4.1
Citrus quinone	12.0	8.4
Co Q_6	12.2	10.1
Co Q_{10}	11.8	9.1
Cytochrome C	19.1	12.6

*Activity, expressed as µl O_2 consumed per minute per mg mitochondria protein, was determined polarographically in 3 ml volumes containing a buffered (pH 7.2) mitochondrial suspension, sodium succinate and the additions.

CITRUS LIMONOIDS: SOME RECENT INVESTIGATIONS

Raymond D. Bennett

WESTERN REGION

Fruit and Vegetable Chemistry Laboratory
Pasadena, California

About 30 years ago the problem of bitterness due to limonin in citrus juices became serious enough to stimulate research into the chemistry of limonoids. Limonin itself had been known for over 100 years, and during the next 20 years the other three major citrus limonoids, nomilin, obacunone, and deacetylnomilin, were isolated. When the structures of these compounds became known, a biosynthetic pathway was proposed: deacetylnomilin ⟶ nomilin ⟶ obacunone ⟶ limonin. However, this sequence required other intermediates which had not been isolated from citrus fruits and this led us to undertake an investigation of minor citrus limonoids. During the course of this work we have isolated several limonoids which may be involved in limonin biosynthesis, and also some other compounds which appear to be metabolites of limonin.

If abacunone is a precursor of limonin, obacunoic acid and isoobacunoic acid are likely intermediates. These compounds had been prepared chemically from obacunone, but at the start of this work neither was known to occur in citrus. We found isoobacunoic acid in grapefruit seeds, but we were unable to detect obacunoic acid. However, we also found two new compounds, nomilinic acid and deacetylnomilinic acid. The latter could be a general precursor of limonoids, producing nomilinic acid by acetylation, deacetylnomilin by lactone ring

closure, or isoobacunoic acid by ether ring closure. Isoobacunoic acid could then be converted to limonin by hydroxylation and lactone ring closure.

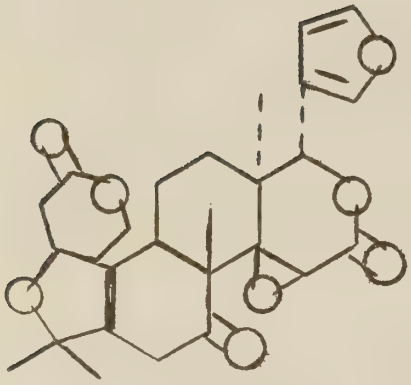
Another pathway could involve ichangin as an immediate precursor of limonin. Ichangin was originally isolated from the rare Ichang lemon, but we have since found it in grapefruit seeds and lemon seeds. Ichangin could be formed from deacetylnomilinic acid by hydroxylation and lactone ring closure, and it could be converted to limonin by ether ring closure. This pathway or the one proceeding from deacetylnomilinic acid via isoobacunoic acid now seems more likely than the one originally postulated, based upon our failure to detect obacunoic acid and the relatively large amounts of obacunone and nomilin which accompany limonin. Biosynthetic intermediates are usually turned over rapidly and therefore are found in low concentrations.

The work of Hasegawa at this laboratory on bacterial metabolism of limonoids led to the discovery of two metabolic pathways. One involved oxidation at C-17 of limonoic acid A-ring lactone (the naturally occurring form of limonin in fruit tissues) to 17-dehydrolimonoic acid A-ring lactone, and the other proceeded by reduction of limonin to deoxylimonin, followed by cleavage of the B-ring to produce deoxylimonic acid. Both 17-dehydrolimonoic acid A-ring lactone and deoxylimonic acid have now been isolated from citrus. Deoxylimonin had been known as a citrus consitutent prior to the microbiological work, but it was considered to be a side product of limonin biosynthesis rather than a metabolite. Our finding of deoxylimonic acid now makes it likely that, as in bacteria, deoxylimonin is the first step in a metabolic pathway.

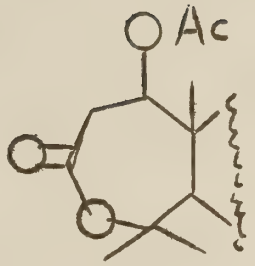
A third possible metabolic sequence is suggested by our finding of a new limonoid, which we have named isolimonic acid to indicate its relationship to deoxylimonic acid. This compound could be derived by oxidative cleavage of the B-ring of limonin. In contrast to deoxylimonic acid, the epoxide group has remained intact.

We have isolated two limonoids, limonol and obacunol, in which the 7-carbonyl has been reduced to a 7α-alcohol. This suggests another possible metabolic pathway, since limonol (which had previously been prepared by chemical reduction of limonin but was not known as a natural product) on treatment with base undergoes rearrangement and loss of the furan ring to produce merolimonol. Only limonoids containing a 7α-hydroxyl group react in this way. Another new citrus limonoid, deoxylimonol, combines this structural feature with loss of the epoxide group associated with the deoxylimonic acid pathway.

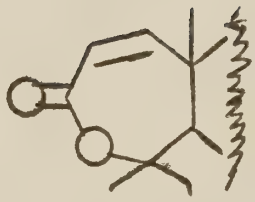
The results of this work have suggested several plausible pathways for the biosynthesis and metabolism of limonin. To find out which are actually operative in citrus fruits, preparation and administration of radioactively labelled limonoids will be necessary.



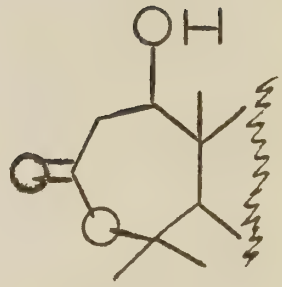
LIMONIN



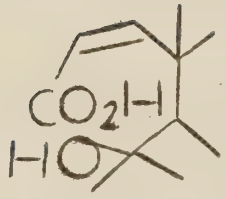
NOMILIN



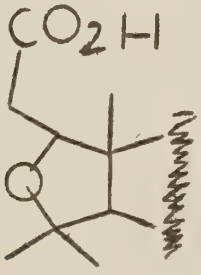
OBACUNONE



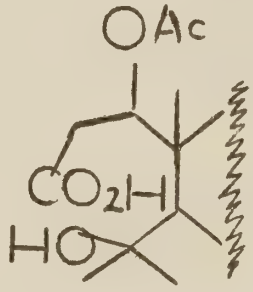
DEACETYLNOMILIN



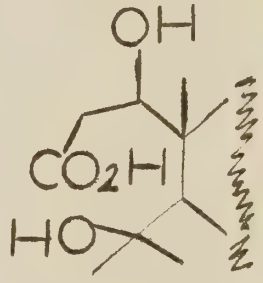
ISOOBACUNOIC ACID



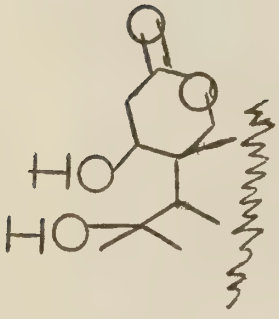
OBACUNOIC ACID



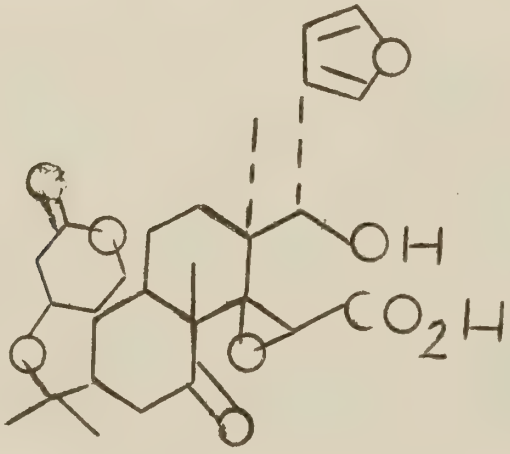
NOMILINIC ACID



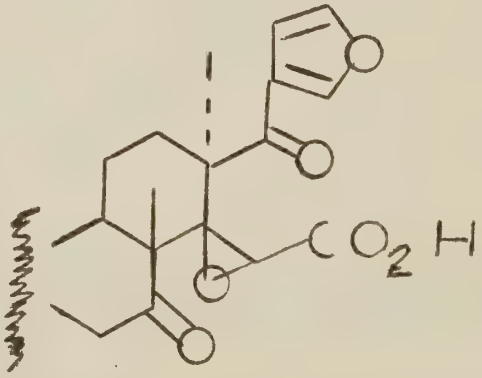
DEACETYLNOMILINIC ACID



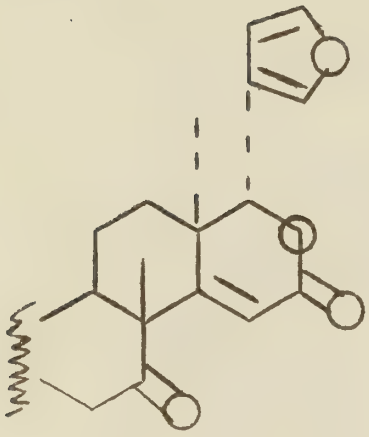
ICHANGIN



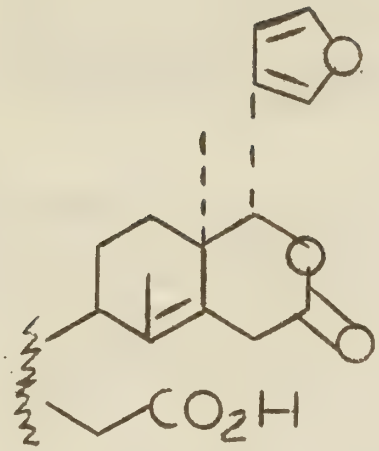
LIMONOIC ACID
A-RING LACTONE



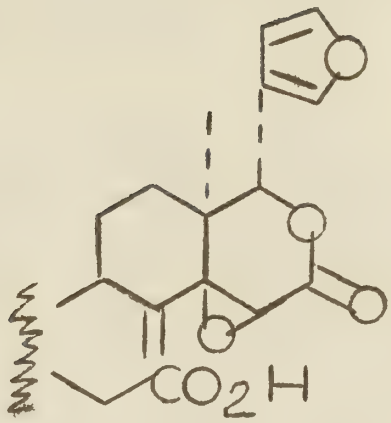
17-DEHYDROLIMONOIC ACID
A-RING LACTONE



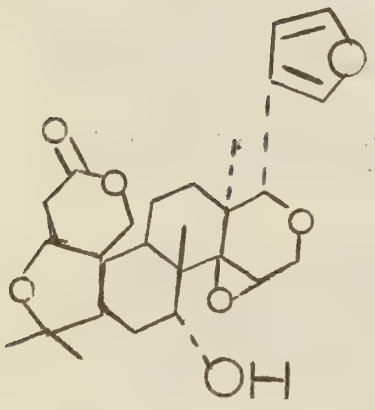
DEOXYLIMONIN



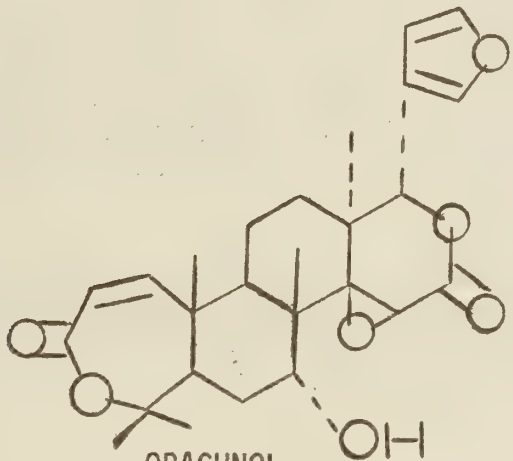
DEOXYLIMONIC ACID



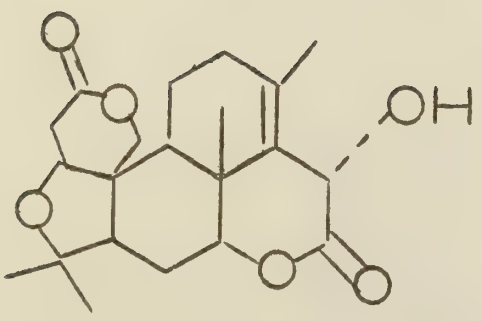
ISOLIMONIC ACID



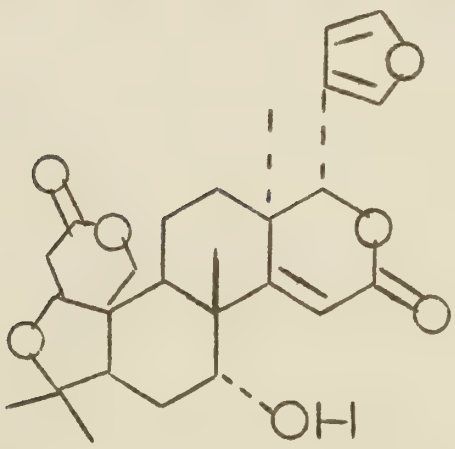
LIMONOL



OBACUNOL



MEROLIMONOL



DEOXYLIMONOL

PROTEIN AND AMINO ACID ANALYSES OF DRY CITRUS SLUDGES FOR POULTRY FEED

Richard L. Coleman and Philip E. Shaw

Citrus and Subtropical Products Laboratory
Winter Haven, Florida

Three sludges found as citrus processing waste by-products were dried and analysed for their crude protein and qualitative and quantitative amino acid contents (see Table 1). The three sludges were: (1) Dried sludge - prepared from a gelatineous material removed from the surface of a clarifier receiving effluent from an aerobic digester used by a commercial citrus processor; (2) Sun dried - sludge or residue which accumulated and dried on the spray field of a commercial processor whose waste system did not provide aerobic and/or anaerobic digestion; (3) Anaerobic residue - sludge formed in the anaerobic digester of a commercial citrus plant and dried for analysis at this laboratory. The qualitative and quantitative amino acid profiles are listed in Table 1 which also includes their total crude protein calculated from Kjeldahl nitrogen.

These sludges have been considered as poultry feed supplements. In tests conducted on a dried citrus sludge comparable to the materials of Table 1, Damron et al. (1974)[1] reported low utilization of sludge protein as a poultry feed supplement. When the amino acids were compared to the amino acid requirements for broilers (see Table 1), certain deficiencies (limiting amino acids) became apparent. These limiting amino acids are listed in Table 2 with the percentage in each sludge sample of that necessary to make a complete protein supplement. From these data a theoretical utilization factor was calculated (Table 3).

The theoretical utilization factor can be improved by addition of certain common materials containing the limiting amino acids. However, feed trials employing these additives will be needed to assess any resulting improved supplement derived from citrus sludge.

LITERATURE CITED

1. Damron, B. L., Eldred, A. R., Angalet, S. A., Fry, J. L. and Harris, R. H. 14th Annual Short Course for the Food Industry, University of Florida, Gainesville, Florida, September 10-12, 1974.

Table 1. Amino acid and protein composition of dried citrus sludge and comparison with amino acid requirements for broilers.

Amino acids	Requirements for broilers[a]	Dried sludge	% of Dried residue Sun dried	% of Dried residue Aerobic
Arginine	1.4	1.32	0.422	1.18
Glycine and/or	1.15	1.62	0.571	1.66
Serine		0.92	0.412	0.92
Histidine	0.46	0.37	0.101	0.31
Isoleucine	0.86	1.13	0.420	1.02
Leucine	1.6	2.11	0.757	1.85
Lysine	1.25	1.25	0.382	1.30
Methionine	0.86	0.67	0.120	0.54
or				
Methionine	0.46	0.67	0.120	0.54
+ Cystine	0.40	NC[b]	NC	NC
Phenylalanine	1.5	0.36	0.104	0.39
or				
Phenylalanine	0.8	1.44	0.425	1.24
+ Tyrosine	0.7	0.80	0.270	0.88
Threonine	0.8	1.34	0.507	1.18
Tryptophane	0.23	0.36	0.104	0.39
Valine	1.0	1.73	0.574	1.51
Alanine	N[c]	2.61	0.923	2.34
Ammonia	N	0.48	0.137	1.19
Aspartic	N	2.74	0.992	2.65
Glutamic	N	3.61	1.084	3.10
Proline	N	1.10	0.450	1.03
Total	-	25.45	8.65	23.8
Calcd. protein[d]	-	42.01	20.94	34.25

[a] National Academy of Science Nutrient Requirements for Poultry, Washington D.C., 1971, Page 15.

[b] NC - not calculated

[c] N - not listed as an essential amino acid in poultry.

[d] From Kjeldahl analysis.

Table 2. Percent of required but limiting amino acids present in citrus sludges.

Amino acid	Dried sludge	Sun dried	Anaerobic
Arginine	94.3	30.1	84.3
Histidine	80.4	21.9	67.4
Methionine and/or Cystine	77.9	14.0	62.8

Table 3. Utilization limiting factors for citrus sludges as poultry feed supplements.

	Dried sludge	Sun dried	Anaerobic
Protein (Kjeldahl)	42.01	20.94	34.25
Available amino acids	25.45	8.65	23.8
Limiting amino acids (Methionine and/or Cystine)	77.9	14.0	62.8
Theoretically utilized amino acids based on available amino acids required for poultry	19.8	1.2	14.9

TEMPERATURE AND STORAGE EFFECTS ON % RETENTION AND % U. S. RDA OF VITAMIN C IN CANNED SSOJ

Steven Nagy and John M. Smoot

Citrus and Subtropical Products Laboratory
Winter Haven, Florida

The retention of vitamin C potency in citrus products is important to the processor and the consumer. Many studies[1-4] have shown that losses of vitamin C in canned single-strength orange juice (SSOJ) are related to storage temperature. High temperatures may result from processing, unfavorable heat dissipation of freshly processed juice (stack-burn), warehouse storage (heat pockets, improper insulation, poor air circulation), poor transit conditions, (over-heated tractor trailers, railway cars, etc.) and poor handling at the supermarket (lack of rotation of flavor-sensitive foods).

The purpose of this study was to determine the effects of high temperatures on retention of vitamin C potency and changes in % U.S. RDA in canned SSOJ. Studies were conducted over a 12-week period on canned juices obtained during the 1972-73 processing season. Figure 1 shows the loss of vitamin C (log % retention) over 12 weeks at 85°, 100° and 115°F. At all 3 temperatures there was rapid loss of vitamin C for the first 2 weeks. This has been shown to be due to free oxygen trapped in the can during processing. After this initial 2 weeks, vitamin C degradation followed an anaerobic decay rate, specific but different for each of the 3 temperatures.

Examination of 14 different juices showed percent retention of vitamin C after 12 weeks storage were:

Temp. (°F)	% Ret. (mean ± S.E.)	Temp. (°F)	% Ret. (mean ± S.E.)
40	99.0 ± 0.3	100	79.1 ± 0.7
60	97.7 ± 0.4	105	70.3 ± 0.8
70	96.3 ± 0.4	110	63.8 ± 0.6
85	92.0 ± 0.6	115	39.1 ± 1.3
90	90.3 ± 0.9	120	5.6 ± 1.2
95	86.5 ± 0.4		

The effects of temperature are even more important when considered for their effects on changing % U.S. RDA values. Where an important nutrient is concerned, i.e. vitamin C, factors which affect its concentration will also affect the nutrient label value (% U.S. RDA) placed on the processed product. Since orange juice from different seasons contains different vitamin C concentrations, the critical factor in nutritional labeling may be the initial vitamin C level. The effects of temperature on vitamin C content expressed as % U.S. RDA values are shown in Table 1. It is evident that juices which initially possess low vitamin C levels are most prone to fall below the 100% U.S. RDA value (60 mg vitamin C/day).

Table 1. Effects of temperatures (°F) on retention of vitamin C

	% U.S. RDA					
	60°	90°	100°	105°	110°	115°
Early-season						
Plant A	142	132	117	109	95	68
B	129	122	107	97	89	58
C	147	137	119	108	97	68
Mid-season						
Plant A	171	156	136	124	108	68
B	145	132	116	103	92	59
C	162	146	131	119	103	61
Early-Valencia						
Plant A	132	120	106	92	83	52
B	116	108	95	86	77	50
C	120	112	99	89	79	51
D	140	130	114	101	92	59
Late-Valencia						
Plant A	114	96	84	74	75	38
B	90	86	75	64	59	30
C	119	110	96	81	75	39
D	112	109	93	80	75	41

LITERATURE CITED

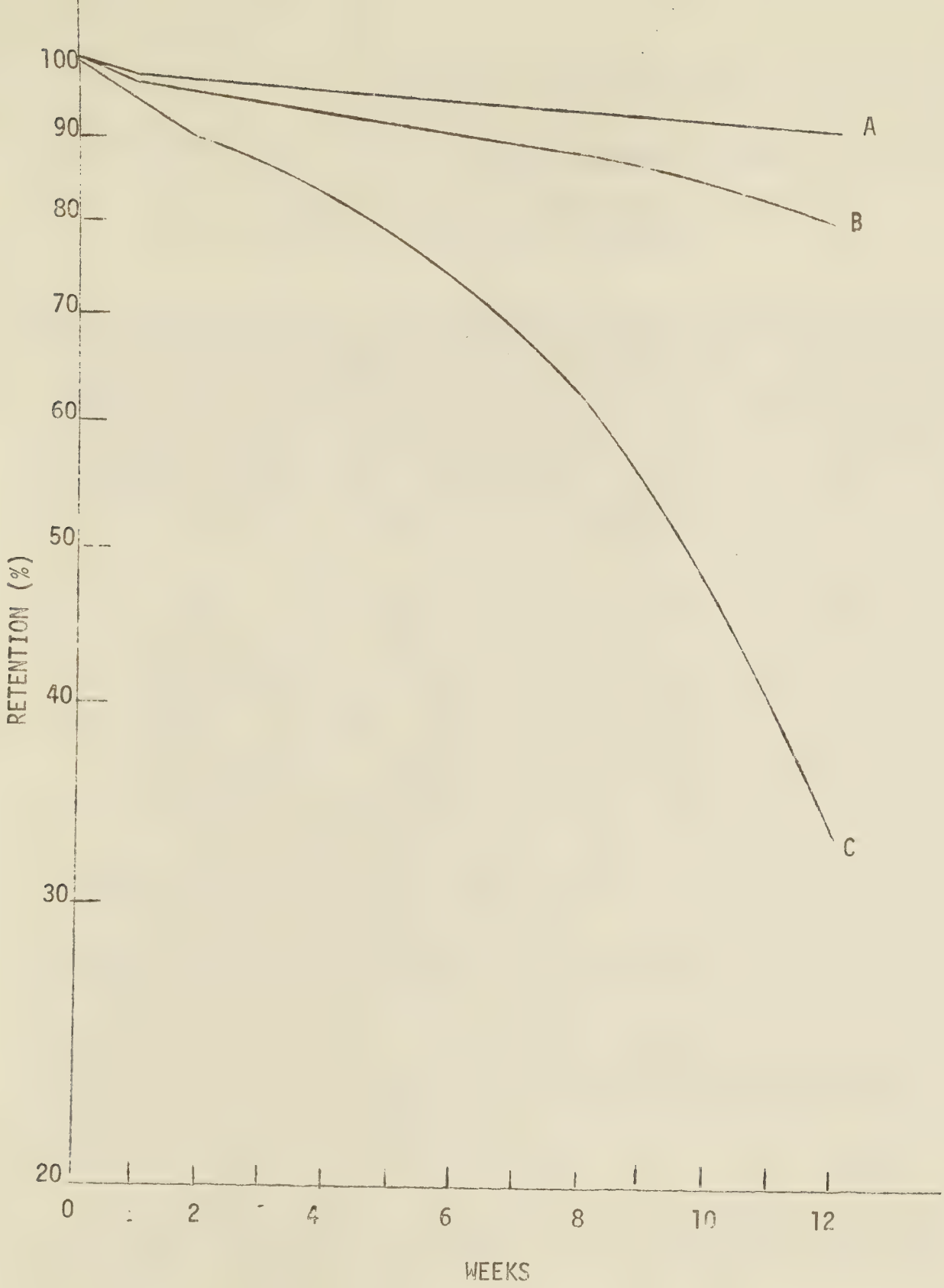
1. Moore, E. L., Wiederhold, E. and Atkins, C. D. 1944. Fruit Products J. 23, 270.

2. Moschette, D. S., Hinman, W. F. and Halliday, E. G. 1947. Ind. Eng. Chem. 39, 994.

3. Sheft, B. B., Griswold, R. M., Tarlowsky, E. and Halliday, E. G. 1949. Ind. Eng. Chem. 41, 144.

4. Blundstone, H. A. W., Woodman, J. S. and Adams, J. B. 1971. In The Biochemistry of Fruits and Their Products, Vol. 2, A. C. Hulme (ed.). Academic Press, New York. p. 543.

(See Figure 1, page 18)


100
90
80
70
60
50
40
30
20
RETENTION (%)
A
B
C
0
2
4
6
8
10
12
WEEKS

GRAPEFRUIT JUICES AND QUALITY

James H. Tatum and Robert E. Berry

Citrus and Subtropical Products Laboratory
Winter Haven, Florida

In 1968 Maier and Beverly ran extensive studies on the various parts from Navel oranges and Marsh grapefruit. They found the non-bitter precursor of limonin in the water extracts of the albedo and carpellary membranes from immature Marsh grapefruit. On acidification the extracts yielded the bitter principle limonin. When these tests were conducted on mature grapefruit (harvested in February) no limonin was found. In 1970 Maier and Grant reported finding 2.2 ppm limonin in mature late-season Marsh grapefruit. In 1972 Tatum, Lastinger and Berry tested single-strength canned grapefruit juice from 11-27-71 to 5-19-72. The limonin content decreased from 10 ppm to about 3 ppm as the season progressed.

Samples of single-strength juice were prepared as follows: (1) light extractions, light finish, (2) heavy extraction, heavy finish and (3) State Test House extraction. These samples were made in July from extremely mature fruit and in August from immature fruit. The late-season juice had less than 2 ppm limonin and was quite acceptable flavor-wise. The early-season immature fruit contained 9 ppm limonin. This juice was bitter and extremely acid. TLC of whole juice showed a vast difference in concentration of most components (especially coumarins and psoralins) between early- and late-season fruit with the early-season showing a much higher concentration of most compounds.

Late bloom mature Marsh grapefruit and early-season Duncan grapefruit were divided into their component parts: juice sacs, rag, albedo and flavedo. These various fractions were examined by thin-layer chromatography and taste tests. These results will be discussed. Consistent differences were noted in the coumarin and psoralins present in extracts from different parts, as well as in the limonin content.

It appears that if the fruit were allowed to mature longer on the trees before processing, quality could be improved.

MECHANICAL REMOVAL OF TRASH AND UNWHOLESOME FRUIT DURING UNLOADING

William L. Bryan, Barry J. Anderson, R. Paul Snyder, Jere M. Miller and James Jenkins

Citrus and Subtropical Products Laboratory
Winter Haven, Florida

A 10-box/min. pilot receiving line was installed at Lykes Pasco Packing Co., to develop economically feasible mechanical methods for removing trash (twigs, leaves, sand, etc.), attached stems and unwholesome fruit while unloading from trucks and conveying to storage bins at commercial rates (up to 60 boxes/min.). Improved mechanization of unloading facilities will reduce labor costs, particularly where the amount of unwholesome exceeds allowable for bin storage (10% unwholesome including 2% decayed). Equipment in the pilot line included:

1) A system of belts, elevators, and chutes for conveying fruit to and from the pilot line and collecting samples. Fruit rate was controlled by unloading through a shallow hopper (2 ft. x 2 ft. opening) positioned 6 in. above a variable-speed conveyor belt.

2) An experimental destemmer designed to grasp and remove attached stems longer than 2 in. (loaned by Gulf Machinery Co.).

3) A 14-brush fruit scrubber (operated dry or wet) to remove adhering sand and leaves.

4) A mechanical grader designed to remove the most degraded unwholesome fruit and less than 20% of the sound fruit into a concentrated stream for more effective manual grading.

The mechanical grader (Figure 1) consisted of: 1) a roller conveyor designed to align fruit into four lanes 5 in. wide operated at 10 valleys/sec. (150 ft./min.), 2) a 5 ft. acceleration ramp inclined 65° with lanes constructed of angle iron to control fruit path from the conveyor, 3) a rotating drum (18 in. diameter) positioned for fruit to strike the surface at an angle 10° below horizontal, and 4) a belt conveyor with partitions to separate fruit by distance projected. Sound fruit were usually projected farther than unwholesome fruit, as previously demonstrated with a flat bounce pad or a circular arc (1). Advantages of the rotating drum compared to a bounce pad or arc were:

1) Projection distance of all fruit could be controlled by adjusting drum rotation speed. This was particularly useful in starting up with fruit of unknown bounce characteristics.

2) Fruit could be graded at higher rates without collisions.

3) The bounce surface could be continuously cleaned with a rotating brush.

The pilot line was operated late in June with six truck loads (3,000 boxes) of hand-picked Valencia oranges at rates of 5-8 boxes/min. with these results:

1) Trash averaged 0.20% of each load with an average distribution of 42% leaves, 35% twigs and stems, and 23% sand. Most trash was removed by the unloading conveyor and the destemmer.

2) Fruit with attached stems varied among loads from 2 to 9%, with about half the stems longer than 2 in. The destemmer removed more than 50% of stems longer than 2 in.

3) Unwholesome fruit varied among loads from 1 to 6% (3% average unwholesome with distribution: 45% drops and stale fruit, 16% split fruit, 34% decayed and 4% decomposed and fruit parts). The mechanical grader was operated at 60-100 rpm drum speed with the partition set at 42-47 in. horizontal distance. About half the unwholesome fruit (including all decomposed) fell short of the partition along with about 5% of the sound fruit. This stream averaged 24% unwholesome fruit (8-fold higher level of unwholesome than in original fruit). Additional unwholesome fruit could be removed by adjusting the grader to separate a larger stream for manual grading.

During the 1975-76 season, the pilot unloading line will be modified to increase capacity to 15-20 boxes/min. and improve effectiveness of the destemmer and grader. The feasibility of 2-stage grading (mechanical regrading) will also be assessed. Both hand-picked and mechanically harvested fruit will be evaluated.

LITERATURE CITED

1) Bryan, W. L., Anderson, B. J. and Norman, G. L. 1974. Proc. Fla. State Hort. Soc. 87, 313.

(See Figure 1, page 22)

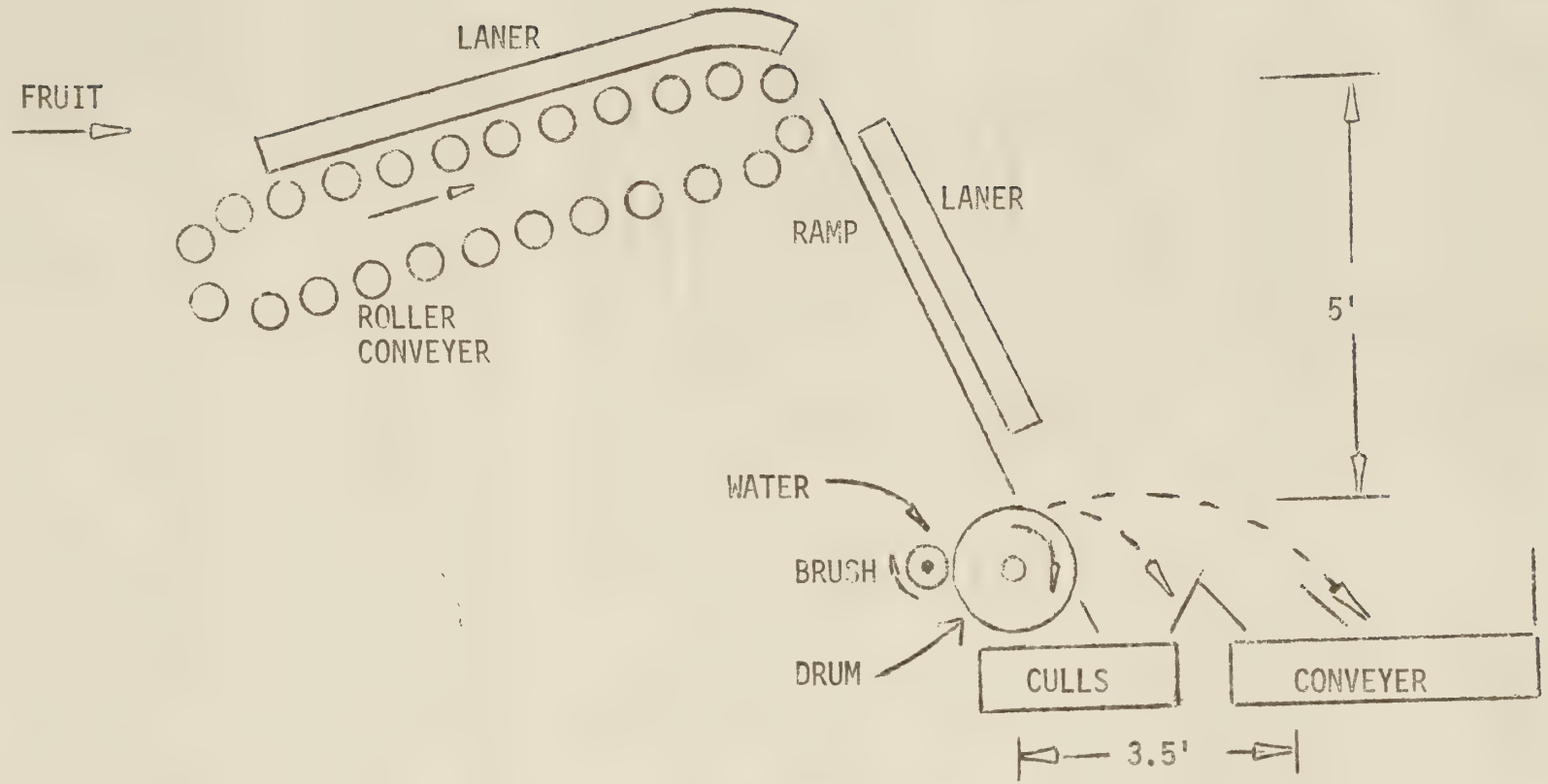


Figure 1. Drum Grader Schematic

ORANGE FLAVOR EFFECTS DUE TO CITRUS ABSCISSION AGENTS

Manuel G. Moshonas, Philip E. Shaw and Donald A. Sims

Citrus and Subtropical Products Laboratory
Winter Haven, Florida

Most of the Florida citrus crop may be harvested mechanically in the future and used in processed products. The anticipated expansion of mechanical harvesting of oranges within the Florida citrus industry introduced a need to assess the effects of mechanical harvesting on product quality. In most mechanical harvesting systems being tested, application of abscission chemicals is required to loosen the fruit prior to mechanical harvesting. Most abscission chemicals function by altering metabolic pathways within the fruit, bringing about release of wound ethylene, and could thus affect formation or balance of flavor components. This study was undertaken to determine the effects of some abscission agents on the flavor quality of processed juice products obtained from early- (Hamlin), mid- (Pineapple) and late-season (Valencia) oranges at different stages of maturity.

The four abscission chemicals employed were those most commonly used in experimental and limited commercial trials by the citrus industry. Trade names of these agents are Acti-Aid, Release, Pik-off and Ethephon. The first three act through damage to the peel which causes the release of wound ethylene to promote abscission. Release and Pik-off are effective in loosening Valencia oranges without damage to young fruit of next season's crop. Ethephon does not damage the peel but breaks down chemically to release the necessary ethylene for abscission. However, Ethephon causes excessive leaf drop, making it a less-desirable agent for mechanical harvesting of fruit to be used in processed products.

Flavor evaluations were made by triangle comparison tests for flavor differences and paired comparison tests for flavor preferences. These tests compared: (1) processed single-strength experimental (abscission-treated) orange juices versus equivalent control (non-treated) juices; (2) experimental orange juice concentrates versus equivalent control concentrates; (3) stored experimental and control processed single-strength orange juices held at 70° and 85°F versus the identical juices held at 0°F.

Juice products from Hamlin, Pineapple and Valencia experimental oranges treated with any of the abscission agents were distinguished from non-treated samples by a trained, experienced panel as shown in Tables 1 and 2. Many of the panel members indicated that the experimental juices had an over-ripe flavor which was considered an adverse effect. In most tests the panel preferred the control juice and in a few cases no preference was established. However, in no test was the experimental juice preferred by the panel.

Since these results were tested for detectable differences by a trained experienced panel, it is uncertain if the general consumer would detect flavor changes in these products or whether such threshold flavor changes would be objectionable. Also, at current usage proportions, mechanically harvested fruit is such a small proportion of total processed fruit, with which it would

be blended, that flavor effects on current products would be negligible. However, the flavor changes observed in this study emphasize the importance of carefully controlling the concentration of these abscission chemicals and of utilizing the minimum time required for fruit loosening after spraying in order to minimize such flavor changes.

Table 1. Flavor evaluation of juice from early- (Hamlin) and mid-season (Pineapple) abscission chemical treated and control oranges

Harvest date	°Brix/Acid ratio	Abscission agent	Concn. ppm	Days on tree	Flavor evaluation confidence level: Difference exp vs con	Flavor evaluation confidence level: Preference for control
Hamlin						
11-25-73	10.45	Acti Aid	20	7	0.05	N.S.[a]
01-04-74	12.86	Acti Aid	20	7	0.001	0.01
Pineapple						
01-25-74	9.87	Acti Aid	20	6	0.001[b]	0.01[b]
03-21-74	17.13	Acti Aid	20	6	0.001	0.01
01-17-75	12.22	Acti Aid	10	6	0.001[b]	0.01[c]
03-11-75	13.95	Acti Aid	10	6	0.001	0.01
01-17-75	13.22	Ethephon	250	6	0.01	N.S.

[a]Not significant at 0.05 confidence level or greater.

[b]For both single-strength juice and concentrate.

[c]Not significant for concentrate.

Table 2. Flavor evaluation of juice from late-season (Valencia) abscission chemical treated and control oranges.

Harvest date	°Brix/Acid ratio	Abscission agent	Concn. ppm	Days on tree	Flavor evaluation confidence level: Difference exp vs con	Flavor evaluation confidence level: Preference for control
3-28-74	13.77	Acti Aid	20	7	0.001	0.01
6-24-74	15.94	Acti Aid	20	7	0.001	0.01
4-02-75	10.44	Release	250	5	0.001	0.01
6-03-75	12.02	Release	250	4	0.001	0.01
4-02-75	10.61	Pik-Off	300	5	0.001	0.01
6-03-75	11.61	Pik-Off	300	4	0.001	N.S.[a]

[a]Not significant at 0.05 confidence level or greater.

UNITED STATES DEPARTMENT OF AGRICULTURE

AGRICULTURAL RESEARCH SERVICE

LIST OF CITRUS PUBLICATIONS

AND PATENTS

(September 1, 1974 - August 31, 1975)

Reprints of publications may be obtained without cost by addressing requests to the Laboratory listed.

Patents may be obtained only by purchase from the U. S. Patent Office, Washington, D.C. 20250, for 50¢ each.

SOUTHERN REGION - FLORIDA ANTILLES AREA

CITRUS AND SUBTROPICAL PRODUCTS LABORATORY
600 Avenue S, N.W.
Winter Haven, Florida 33880

QUANTITATIVE COMPOSITION OF COLD-PRESSED ORANGE OILS
Philip E. Shaw and Richard L. Coleman
J. Agr. Food Chem. 22(5), 785-787 (1974).

RELATIONSHIP OF FURFURAL TO TEMPERATURE ABUSE AND FLAVOR CHANGE IN COMMERCIALLY CANNED SINGLE-STRENGTH ORANGE JUICE
Steven Nagy and Howard L. Dinsmore
J. Food Sci. 39, 1116-1119 (1974).

FATTY ACIDS OF TRIGLYCERIDES AND STEROL ESTERS FROM DUNCAN GRAPEFRUIT, DANCY MANDARIN AND THEIR TANGELO HYBRIDS
Harold E. Nordby and Steven Nagy
Phytochem. 13, 2215-2218 (1974).

ULTRAVIOLET ABSORPTION METHOD FOR EVALUATING CITRUS ESSENCES
J. M. Randall, W. L. Bryan, O. W. Bissett and R. E. Berry
J. Food Sci. 38, 1047-1050 (1973).

ASCORBIC ACID RETENTION IN ORANGE JUICE AS RELATED TO CONTAINER TYPE
Owen W. Bissett and Robert E. Berry
J. Food Sci. 40, 178-180 (1975).

ESTIMATION OF ASCORBIC ACID IN ORANGE JUICE BY A CHRONOMETRIC METHOD
Bongwoo Roe and Joseph H. Bruemmer
Proc. Fla. State Hort. Soc. 87, 210-213 (1974).

ANALYSIS OF CONCENTRATED ORANGE ESSENCE AND COMPARISON WITH KNOWN ESSENCE COMPOSITION
Philip E. Shaw and Manuel G. Moshonas
Proc. Fla. State Hort. Soc. 87, 305-310 (1974).

MECHANICAL GRADING OF ORANGES BASED ON DYNAMIC BEHAVIOR
William L. Bryan, Barry J. Anderson and Gary L. Norman
Proc. Fla. State Hort. Soc. 87, 313-318 (1974).

CHARACTERIZATION OF CITRUS CULTIVARS AND SEPARATION OF NUCELLAR AND ZYGOTIC SEEDLINGS BY THIN LAYER CHROMATOGRAPHY
James H. Tatum, Robert E. Berry and C. Jack Hearn
Proc. Fla. State Hort. Soc. 87, 75-81 (1974).

THE RELATIONSHIP OF LONG-CHAIN HYDROCARBONS TO THE CHEMOTAXONOMY OF CITRUS
Harold E. Nordby and Steven Nagy
Proc. Fla. State Hort. Soc. 87, 70-74 (1974).

BIOCHEMICAL CHANGES IN GRAPEFRUIT STORED IN AIR CONTAINING ETHYLENE
Paul L. Davis, Bongwoo Roe and Joseph H. Bruemmer
Proc. Fla. State Hort. Soc. 87, 222-227 (1974).

LIPID COMPOSITION OF COMMERCIALLY CANNED SINGLE-STRENGTH ORANGE JUICE
Steven Nagy, Harold E. Nordby and John M. Smoot
JAOCS 52(4), 121-123 (1975).

SATURATED AND MONO-UNSATURATED LONG-CHAIN HYDROCARBON PROFILES FROM CITRUS UNSHIU JUICE SACS
Harold E. Nordby and Steven Nagy
Phytochem. 14, 183-187 (1975).

DEGRADATION PRODUCTS FORMED IN CANNED SINGLE-STRENGTH ORANGE JUICE DURING STORAGE
James H. Tatum, Steven Nagy and Robert E. Berry
J. Food Sci. 40, 707-709 (1975).

PERESTER OXIDATION OF (+)-LIMONENE TO (-)-CARVONE AND PIPERITENONE
Charles W. Wilson and Philip E. Shaw
J. Agr. Food Chem. 23(4), 636-638 (1975).

TWO SIMPLE METHODS FOR EVALUATING CITRUS ESSENCE: BROMATE TITRATION AND GAS CHROMATOGRAPHY
Manuel G. Moshonas and Philip E. Shaw
Int. Flavours 6(2), 133-134 (1975).

COMPOSITION AND FLAVOUR EVALUATION OF A VOLATILE FRACTION FROM A COLD-PRESSED VALENCIA ORANGE OIL
Philip E. Shaw and Richard L. Coleman
Int. Flavours 6(3), 190 (1975).

Fruit and Vegetable Chemistry Laboratory
263 South Chester Avenue
Pasadena, California 91106

CAROTENOGENESIS IN PHYCOMYCES
Wan-Jean Hsu, D. A. Ailion, and M. Delbruck
Phytochem. 13, 1463-1468 (1974).

METABOLISM OF LIMONOIDS, ISOLATION AND CHARACTERIZATION OF DEOXYLIMONIN HYDROLASE FROM PSEUDOMONAS
Shin Hasegawa, V. P. Maier, S. N. Border and R. D. Bennett
J. Agr. Food Chem. 22, 1093-1096 (1974).

SOME FACTORS AFFECTING SENSORY THRESHOLDS AND RELATIVE BITTERNESS OF LIMONIN AND NARINGIN
D. Guadagni, V. P. Maier and J. H. Turnbaugh
J. Sci. Fd. Agric. 25, 1199-1205 (1974).

EFFECT OF SUBTHRESHOLD CONCENTRATIONS OF LIMONIN, NARINGIN AND SWEETENERS ON BITTERNESS PERCEPTION
D. Guadagni, V. P. Maier and J. H. Turnbaugh
J. Sci. Fd. Agric. 25, 1349-1354 (1974).

APIGENIN 4'-O-β-D-GLUCOSIDE 7-O-β-D-GLUCURONIDE: THE COPIGMENT IN THE BLUE PIGMENT OF CENTAUREA CYANUS
S. Asen and R. M. Horowitz
Phytochem. 13, 1219-1223 (1974).

CAROTENOID BIOSYNTHESIS IN RHODTORULA GLUTINIS
Ernest P. Hayman and Henry Yokoyama
J. of Bacteriol. 120, 1339-1343 (1974).

MULTIPLE AUTOMATED ANALYSES FOR ORANGE JUICE CONTENT: DETERMINATION OF TOTAL SUGARS, REDUCING SUGARS, TOTAL ACIDITY, TOTAL AMINO ACIDS, AND PHENOLICS
Carl E. Vandercook, Ruth L. Price, Christina A. Harrington
JAOAC 58, 482-487 (1975).

LIMONOATE DEHYDROGENASE AND PREPARATION THEREOF
Shin Hasegawa and Linda C. Brewster
U.S. Patent No. 3,869,345. Patented March 4, 1975.

DIHYDROCHALCONE GALACTOSIDES AND THEIR USE AS SWEETENING AGENTS
Robert M. Horowitz and Bruno Gentili
U.S. Patent No. 3,876,777. Patented April 8, 1975.

DIHYDROCHALCONE GALACTOSIDES AND THEIR USE AS SWEETENING AGENTS
Robert M. Horowitz and Bruno Gentili
U.S. Patent No. 3,890,296. Patented June 17, 1975.

METHOD ENHANCING COLORATION OF FRUITS AND VEGETABLES WITH A DIALHYLAMINO-ALKOXYBENZENE

Henry Yokoyama, Wan-Jean Hsu and Stephen Poling
U.S. Patent No. 3,864,501. Patented February 4, 1975.

SOUTHERN REGION

Food Crops Utilization Research Unit
Weslaco, Texas 78596

BLENDING TO IMPROVE QUALITY OF TEXAS ORANGE JUICE PRODUCTS

Robert R. Cruse and Bruce J. Lime
J. Rio Grande Valley Hort. Soc. 28, 154-163 (1974).

NOTES: